

Non-DO Estuary Nutrient Endpoints

Draft Report

Submitted to

The Delaware River Basin Commission



By

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Summary

The Delaware River Basin Commission (DRBC) has proposed using the Delaware Estuary Eutrophication model to propose criteria for ambient nitrogen and phosphorus in the Delaware Estuary, as needed, to support dissolved oxygen (DO) endpoints. Under this proposal, nitrogen and phosphorus criteria would be driven by DO endpoints. However, stakeholders have pointed out that there could be other important ecological impacts of elevated nutrients that are not tied to DO. Thus, DRBC has tasked the Academy of Natural Sciences of Drexel University (ANSDU) with querying literature and experts with a goal of developing a limited prioritized list of non-DO estuary nutrient endpoints that could inform nutrient criteria selection.

Extensive literature on direct nutrient effects for species present in the Delaware River and Estuary or from analogous large river systems is largely lacking. And, in general, far more literature was available pertaining to indirect effects of nutrients than direct effects. As a result, robust, quantitative nutrient endpoints relevant to the Delaware Estuary were difficult to determine.

Nitrogen and phosphorus have both been shown to exhibit toxic effects on fish, although increased salinity ameliorates the negative impacts of nutrients on fishes. The limited number of studies investigating lethal effects of nutrients on fish species had mixed conclusions due, in part, to the confounding effects of system size, light, trophic interactions, and water chemistry. Fish community health, as assessed using Indices of Biotic Integrity (IBIs), has been shown to be negatively correlated to nutrients, at concentrations of 1.37 mg/L total inorganic nitrogen and 0.17 mg/L total phosphorus in low-order, freshwater streams. More evidence exists for indirect effects of nutrients on fishes through changes in system productivity and/or changes in abundances of different groups of primary producers (macrophytes, diatoms, green algae, blue-green algae, etc.). However, definitive statements about these bottom-up nutrient effects cannot be made because of the multiplicity of trophic pathways from primary producers to fish.

Nutrients negatively affect the physiology of freshwater shellfish species at a variety of life stages. Endocrine disruption by elevated nutrient levels was of particular problem at all life stages. A few studies suggest thresholds where freshwater macroinvertebrate populations change, although only one took place in large rivers within similar climatic conditions. Studies spanning different river and stream sizes find fewer relationships between macroinvertebrate communities and metrics in large rivers than in small streams. The majority of literature pertaining to nutrient impacts on invertebrate communities showed indirect effects on the invertebrate community in both estuarine and freshwater systems, through algal blooms and resulting oxygen fluctuations, algal mats as enhancing and also by degrading habitat quality, or food web interactions through changes in the algal and macrophyte community.

Overall, consistent, quantified nutrient endpoints associated with algae communities and species are lacking for a wide range of aquatic systems, especially estuaries, and there is a general lack of evidence for nutrient endpoints for specific species. In streams, phosphorus is the main nutrient found to control production leading to eutrophication events, whereas in estuaries nitrogen has a larger influence than phosphorus. Reported concentrations endpoints associated with changes in freshwater algae taxonomy, trophic status, productivity, and/or occurrence of nuisance species varied, highlighting the difficulties in translating endpoints determined for one region to another such as the Delaware River and Estuary. In estuaries, the impacts of nitrogen species have been shown to both increase and decrease with salinity.

Background

The Delaware River's past has been beset by bouts of water quality problems since the late 1800s. By the mid-1900s, population growth and increased wastewater discharge created deleterious conditions in the Delaware River Estuary (Trenton, NJ to the bay) that affected both wildlife and human health. The pollution problems culminated in the creation of the Delaware Estuary Comprehensive Study under the provisions of the Federal Water Pollution Control Act in 1961 to "provide a blueprint for the enhancement of the waters of the Delaware" as well as the establishment of the Delaware River Basin Commission.

In 1998 the U.S. Environmental Protection Agency initiated a program to identify the effects from nutrient alterations, to abate and perhaps reverse the increases in nutrients seen in many surface waters, and to restore the ecological integrity of those waters where excess or altered nutrient regimes were causing impairments to the structure and function of these biological systems. Central to these efforts is the development of numeric nutrient criteria in a manner consistent with the Clean Water Act. The DRBC serves as the lead coordinating agency among the U.S. EPA and the states of New York, New Jersey, Pennsylvania, and Delaware for determining the nutrient criteria or nutrient-related criteria, if appropriate, that are needed to protect aquatic life, public & industrial water supplies, and recreational uses of these shared resources.

In the current version of its nutrient criteria development plan, DRBC has proposed using the Delaware Estuary Eutrophication model (under development) to develop and propose criteria for ambient nitrogen and phosphorus in the Delaware Estuary, as needed. Under this proposal, the model would help define the ambient concentrations and species of nitrogen and phosphorus necessary to achieve dissolved oxygen (DO) targets (also under development). Thus, under this approach, the nitrogen and phosphorus criteria would be driven by DO endpoints. Some stakeholders have pointed out, however, that there could be other important impacts of elevated nutrients that are not tied to DO. These stakeholders have expressed concern that setting nutrient criteria based solely on DO may not address other estuary ecological impacts from nutrients.

As such, ANSDU was tasked with querying literature and experts with a goal of developing a limited prioritized list of non-DO estuary nutrient endpoints that could inform nutrient criteria selection. Focus was given to nitrate and phosphorus species, as separate efforts on ammonia ambient toxicity recommended criteria are ongoing. Characteristics of non-DO nutrient endpoints that would increase their value in setting criteria would include measurable effect(s), high likelihood of being relevant in the Delaware Estuary, and sufficient current knowledge to inform nutrient criteria concentration selection during the period of model development and DO criteria selection. In the absence of information meeting these characteristics, the best available information was summarized as were the associated key data gaps pertaining to non-DO nutrient impacts.

Objective

The objective of this report is to evaluate impacts of nutrients not tied to DO and develop a limited prioritized list of associated estuary nutrient endpoints that could inform nutrient criteria selection.

Methodology

Literature searches were conducted in Google Scholar, Web of Science, Academy of Natural Sciences Archives, and Drexel University libraries to identify potential impacts of elevated nutrients that are not tied to DO and concentration endpoints associated with these impacts. Literature was also obtained by finding references within found literature and by finding literature that has since cited primary literature. The literature, information, and data sought throughout were from appropriate scientific or published sources, and special effort was made to identify and obtain the most recent and reliable studies and reports. All sources consulted have been appropriately documented and listed in this report.

In our search for potential impacts, particular focus was given to possible non-DO nutrient endpoints suggested by DRBC stakeholders and to studies from the Delaware Estuary and comparable, regional systems. We also considered non-regional studies relevant to the Delaware Estuary based on similarities in species, nutrient and salinity concentrations (Table A1) and/or system size. Data on endpoints and threshold concentrations reported in as quantifiable format as possible. Gaps in available information were also identified.

Delaware Estuary Zones

Note: The following information has been compiled from information provided by the DRBC website (<http://www.state.nj.us/drbc>).

The Delaware Estuary has been designated into five zones, numbered 2 through 6, by the DRBC (Fig. 1). Zone 2 extends from the head of tidewater at river mile 133.4 to 108.4 (~Trenton, NJ to Tacony, PA) and has salinities in the range of 0.06 to 0.25‰. Zone 3 extends from river mile 108.4 to 95.0 (~Tacony to South Philadelphia, PA) and exhibits similar salinities. Zone 4 extends from river mile 95.0 to 78.8 (~South Philadelphia to Marcus Hook, PA) and has fresh and oligohaline waters with salinities ranging from 0.10 to 1.30‰. Zone 5 extends from river mile 78.8 to 48.2 (~Marcus Hook to Odessa, DE) and has salinities in the range of 0.10 to 14.7‰. Zone 6 extends from river mile 48.2 to 0.0 (~Odessa, DE to Atlantic Ocean) and ranges in salinity from 0.14 to 31.6‰. The Atlantic Ocean has a salinity of 35+ ‰. The salt line, which is defined as the location of the seven-day average chloride concentration of 250 mg/l, for the estuary is tied closely to river discharge and varies by day and season. As discharge in the river is regulated upstream of the estuary by several reservoirs, the salt line is typically found just south of Wilmington, DE in Zone 5. With periods of severe drought, however, the salt line moves upstream and was at a record of river mile 102, north of Philadelphia, in 1963.



Figure 1. Map of the Delaware Estuary and Delaware River Basin Commission Water Quality Zones. (From: <http://www.state.nj.us/drbc/basin/map/>)

Fish Impacts and Endpoints

Direct Effects

The direct effects of nutrients on fish species and communities are not as readily available in the literature as indirect effects, such as algal blooms or oxygen depletion. However, a handful of studies have examined lethal effects of nitrogen and phosphorus on different fish species with mixed conclusions (Table F). Despite the disparities in studies and results, nitrogen, as either nitrates or nitrites, and phosphorus have been shown to exhibit toxic effects on fish, especially in low salinity waters (Camargo and Alonso 2006; Miltner and Rankin 1998; Russo and Thurston 1977; Weirich et al. 1993).

Several studies have examined the effects of nutrients on fish communities using Indices of Biotic Integrity (IBIs) in wadable streams. These studies have found negative correlations with nutrients (Miltner and Rankin 1998; Wang et al. 2007). Using IBIs and habitat models, Miltner and Rankin (1998) found a significant negative correlation between total inorganic nitrogen (at 1.37 mg/L) and total phosphorus (at 0.17 mg/L) and biotic integrity of fish communities in low-order, freshwater streams. In wadeable streams in Wisconsin, percentages of carnivorous, intolerant, and omnivorous fishes, IBIs, and salmonid abundances were found to be significantly correlated ($p < 0.05$) with at least one nutrient measure (Wang et al. 2007). Specifically, canonical correspondence suggested that nutrients explained 15% of the variation in fish assemblages (Wang et al. 2007).

For individual fish species, salmonids have proven to be highly sensitive to nitrates and nitrites (Camargo and Alonso 2006; Russo and Thurston 1977; Wang et al. 2007). Rainbow Trout (*Oncorhynchus mykiss*) have been shown to have heightened sensitivities to nitrate exposure with 30-day LOECs (lowest significant tested concentration) of 2.3-7.6 mg NO₃-N/L and 30-day NOECs (no observed effect concentration) of 1.1-4.5 mg NO₃-N/L (Camargo and Alonso 2006). Additionally, it has been reported that *O. mykiss* exhibits 96-hour LC50s of 0.14-0.41 mg NO₂-N/L in freshwater and up to 12.5 mg NO₂-N/L in water with 40.8 mg/L of salt, suggesting the addition of chloride ameliorates the effects of nitrites (Russo and Thurston 1977). It has similarly been reported that 50% mortality of *O. mykiss* occurs at 0.23-0.55 mg NO₃-N/L within 24 to 96 hours (Smith and Williams 1974 and Brown and McLeay 1975 as cited in Russo and Thurston 1977). Camargo and Alonso (2006) also reported 96-hour LC50s of 0.1-0.4 mg NO₂-N/L, 0.5-0.6 mg NO₂-N/L for *Oncorhynchus mykiss* and *Salmo clarki*, respectively. In the Delaware Estuary, salmonids are absent, transient, or derived from stocking.

Among other species, Brook Stickleback (*Culaea inconstans*) has shown mortality in 3-5 hours at 5 mg NO₃-N/L; Carp (*Cyprinus carpio*) has shown mortality at 100 mg NO₃-N/L within 45 hours, but no mortality after 48 hours at 40 mg NO₃-N/L; Logperch exhibited mortality in less than 3 hours at 5 mg NO₃-N/L; Spotfin Shiner (*Cyprinella spiloptera*), Johnny Darter (*Etheostoma nitrum*), Bluegill (*Lepomis machrochirus*), Pumpkinseed (*Lepomis gibbosus*), Northern Hog Sucker (*Hypentelium nigricans*) and Stonecat (*Noturus flavus*) have all survived less than 12 to 24 hours in 20-40 mg NO₃-N/L (McCoy 1972 as cited in Russo and Thurston 1977). Fathead minnow (*Pimephales promelas*) has shown sensitivities to nitrite exposure with 96-hour LC50s of 2.3-3.0 mg NO₂-N/L (Camargo and Alonso 2006). Sunshine Bass, which is a hybrid of White Bass (*Morone chrysops*) and Striped Bass (*Morone saxatilis*), has been shown to be sensitive to both ammonia and nitrite with a 96-hour LC50

of 12.8 ± 1.6 mg NO₂-N/L in freshwater, 35.0 ± 2.3 mg NO₂-N/L at a salinity of 1 g/L, and >100 mg/L nitrite-N at salinities of 8 g/L or higher (Weirich et al. 1993). Several of these species (Carp, Spotfin Shiner, Bluegill, Pumpkinseed and Fathead Minnow) or congeners of these species (Tessellated Darter, Margined Madtom, White Perch and Striped Bass) occur in parts of the Delaware Estuary. We have found no studies on the majority of mainly estuarine species which occur in the Delaware Estuary.

Exposure to nitrite has also been shown to cause an increase in methemoglobin concentration in fish with differences observed among species sensitivities (Russo and Thurston 1977). After a literature review, Dodds and Welch (2000) suggested a limit of 0.02 mg/L NH₃ to avoid chronic toxicity. Others have suggested total nitrogen (TN) levels less than 0.5-1.0 mg/L could prevent acidification and eutrophication. (Camargo and Alonso 2006).

Laboratory experiments yield insight into effects of nitrates, nitrites, and phosphorus toxicity; however, it is cautioned that baseline values for N, P, external C supply, and light need to be established when determining how nutrients effect trophic states as interactions are possible and likely (Dodds 2007). Additionally, it has been demonstrated that increased salinity ameliorates the deleterious effects of nutrients on fishes and interactions between chloride and nutrients need to similarly be considered (Weirich et al. 1993).

Indirect Effects

Indirect effects of nutrients on fishes are likely through changes in system productivity and/or changes in abundances of different groups of primary producers (macrophytes, diatoms, green algae, blue-green algae, etc.). Because of the multiplicity of trophic pathways from primary producers to fish, definitive statements about these bottom-up nutrient effects cannot be made. These interactions have been most studied in lakes (e.g., Persson, et al. 1992, Scheffer 1998, Carpenter 1987), and application to the Delaware River estuary and Delaware Bay would be difficult. No attempt was made to review the large literature on productivity and food webs.

While these studies do demonstrate possible lethal effects of nutrients on fishes and fish communities, there still exist gaps in the existing knowledge and literature on direct effects of nitrogen and phosphorus. It is to be noted that for this task order, a preliminary examination of the existing literature was conducted, and several sources mentioned here within have been provided as found within other review papers. These sources have not yet been obtained to determine accuracy of reported results but could be examined in further detail should a more extensive literature review be ordered in a subsequent task order. These sources are appropriately noted within the text and Table A2.

Macroinvertebrate Impacts and Endpoints

The majority of literature pertaining to nutrient impacts on invertebrate communities showed indirect effects, through algal blooms and resulting oxygen fluctuations, algal mats as enhancing and also by degrading habitat quality, or food web interactions through changes in the algal and macrophyte community. These alterations to the ecosystem were connected to changes in the invertebrate community through dominance of a few taxa (Hale 2018, Jones 2006), reduced functional diversity (Hale 2018, Lawes 2018), and replacement of existing fauna with diverse traits by small bodied, short

life cycle populations (Hale 2018, Bierschenk et al. 2017) in both estuarine and freshwater systems. We found evidence of negative nutrient effects on physiology of freshwater shellfish species at a variety of life stages. Endocrine disruption by elevated nutrient levels was of particular problem at all life stages, with reduced embryo production, recruitment, glochidia stage and metamorphosis success, and respiration and filtration in adult stages. A few papers suggested thresholds where freshwater macroinvertebrate populations changed, although only one took place in large rivers within similar climatic conditions (Sunderman et al. 2015; Smith & Tran 2010, Kail et al. 2012; Table A3). Similarly, studies spanning different river and stream sizes find fewer relationships between macroinvertebrate communities and metrics in large rivers than in small streams (Schafer et al. 2016; Paisley et al. 2011).

Freshwater non-shellfish invertebrates

A few laboratory studies and field surveys provided information with specific values for changes in the invertebrate community (Table A3) and may be of value in the Delaware Basin. Perhaps most relevant, in major rivers in New York State, Smith & Tran (2010) found significant changes in the Nutrient Biotic Index, % mesotrophic, % eutrophic individuals relative to total phosphorus (TP) and total nitrogen (TN), in the Nutrient Biotic Index (NBI) and the algae-based Pollution Tolerance Index (PTI) relative to nitrate, and in the Hilsenhoff Biotic Index relative to Chlorophyll -a. The threshold value for significant variables are presented in the table taken from (Smith & Tran, 2010; Table A4). This study notes that if TP concentration fall at or above the 50th percentile, the waterway would be deemed impaired (added to the 303(d) list). An important point that this study makes is that many IBI metrics are not suited to specifically detect or show impairment or stress from nutrients, which is the rationale for their use of the metrics described and in Table A4. One strength of this study is the combination of indices based on periphytic diatoms (PTI, % eutrophic, mesotrophic) and macroinvertebrates (HBI, NBI). It is the best regional study examining nutrient effects on in-stream metrics, considering the metrics used for such assessments as well as conditions that may be comparable to the Delaware Watershed. However, the study does not apply to tidal rivers or a salinity gradient, and should be used as a general reference, not for exact values that can be applied to the tidal Delaware River. The presence of this one study that is highly relevant but not perfectly transferable is evidence that these studies are lacking in most regions, making it difficult to recommend specific thresholds in this literature review. The information available provides insight into the gaps that may need to be filled in with empirical data from studies specifically designed to answer the question put forth in this Task order.

In a study in German streams, Sunderman et al. (2015) found responses of specific invertebrate taxa to levels of various chemical components, including nitrogen and phosphorus compounds (Table A3). Building on the work of Sunderman, also in Germany, Leps et al. (2016) described change points at the 90th percentile for several water chemistry components (10th percentile for dissolved oxygen) in order to quantify the effects of exposure to high concentrations on macroinvertebrate communities. Berger et al. (2018) found change points for several traits related to nitrite, nitrate, and PO₄. The traits that responded to nitrate included life cycle length, adult modality of aquatic stage, (i.e. air breathers, typically), and some other characteristics of highly pollution-tolerant taxa. Due to differences in taxa and their associated metric values, the results of these studies can not be directly applied to the Delaware River and Estuary, however, a similar analysis could be done for the Delaware River and its tributaries to test for effects of certain nutrient levels. The Academy has begun this work as part

of the Delaware River Watershed Initiative. To date, it appears macroinvertebrate taxa respond better to the gradient of water chemistry, habitat and land use combined rather than directly to nutrients or other individual parameters.

Few mesocosm or laboratory experiments have been performed, but focusing on toxicity levels, they found effects on net spinning caddisflies at concentrations 10-20x higher than typical ambient conditions in the estuary, reinforcing other research that nitrate and other nutrients have more severe effects through indirect pathways (Camargo et al. 2005; Camargo & Ward 1992).

Using presence-absence macroinvertebrate data in Danish rivers, Friberg et al. (2010) found negative responses to total phosphorus and ammoniacal nitrogen, but no relationship with total nitrogen. The authors concluded, like many others, that macroinvertebrates are poor indicators of eutrophication. This may have a result of their data type (presence-absence) and local conditions (Paisley et al. 2011).

One main limitation of the reviews for freshwater may, in fact, be the metrics used, as Smith & Tran (2010) note. Schäfer et al. (2016), Paisley et al. (2011), and Friberg et al. (2010) posit that macroinvertebrates may be a poor indicator of nutrient enrichment. Although they may not be as strong an indicator as diatoms (Hering et al. 2006), use of macroinvertebrates for their response to nutrients may be stronger by studies analyzing with metrics specifically tailored to nutrients or to life history and ecological traits.

In setting nutrient criteria, it may also be important to consider several papers that linked declines in macroinvertebrate community integrity (as defined by lower IBI scores, reductions in certain Functional Feeding Groups and other metrics) to sediment concentrations, which may be another indirect pathway or a confounding factor and covariate of nutrient-induced stress, as phosphorus is known to bind to sediments and the degree of nitrogen-compound binding is not as well documented. As many authors have noted, it is difficult to parse out effects of multiple stressors (Berger et al. 2018; Vinebrook et al. 2004, Wagenhoff et al. 2012). One study worth considering is Bierschenk et al. (2017) on the effects of catchment land use on invertebrates along a freshwater-marine gradient. In general, the authors observed reduced abundance from freshwater to estuarine to near-marine sites, which is expected, and may influence assessment of integrity in the Delaware basin relative to salinity levels. Although we have no doubt that the DRBC is aware of this pattern, it is one factor to keep in mind when assessing ecological integrity as salinity rises downstream. This study was one of many that found fine sediment to be the best predictor of changes in traits in the macroinvertebrate community, which may be related to nutrient effects. The authors did not find different levels of effects of land use (and associated sediment and nutrients) along the salinity gradient. As one metric in the DRBC IBI, the authors did find a negative relationship between scrapers/grazers and fine sediment.

Freshwater shellfish invertebrates

In our review of nutrient effects on freshwater shellfish invertebrates, we found both lethal and sublethal outcomes for a variety of life stages of numerous species. In an overview of ecological effects of freshwater pollution, Harmon and Wiley (2012) noted chronic sensitivity to ammonia in early life-stages of mussels at levels below the EPA's criteria for the pollutant. They also cite a study that mussels in wastewater aeration lagoons showed neuroendocrine disruption, among other health

effects. Ammonia concentrations between 0.5-1.5 mg/L were found to have significant impacts on survival, growth and feeding rates of a freshwater river prawn in another study cited.

In laboratory experiments studying sodium phosphate toxicity to quagga mussels, Vijayal and Kashian (2019) found increasing mortality with increased exposure time and concentration, which was associated with inhibitory action by sodium phosphate on oxygen consumption by damaging respiratory epithelium and ultimately their gills. They also found sublethal effects on physiology and feeding, associated with inhibitory action by sodium phosphate on ciliary action of mussels, mucus secretion in response to the toxicant clogging their siphons leading to low filtration (Sze and Lee 2000), as well as decreased respiration and feeding rates due to shell closure when exposed to toxicants.

As indicated, early life stages of larger bodied freshwater macroinvertebrate species can be particularly sensitive to nutrient enrichment. Moore and Bringolf (2018) studied nitrate effects on the parasitic glochidia phase of two freshwater river mussel species. While treatments did not affect glochidia viability after 24 hours of exposure to nitrate concentrations up to 200 mg/L, there was an 11% and 35% reduction (both statistically significant) in total juveniles produced for *Lampsilis siliquoidea* at 11 mg/L and 56 mg/L NO₃-N, respectively, however there was no apparent effects for *Lampsilis fasciola*, which was at least partially attributed to the older age of these glochidia during the study period. They attribute reduced juvenile success to decreases in both glochidia attachment to host gills, and success in metamorphosing after attachment, due to endogenous conversion of nitrate to nitrous oxide acting as a source of endocrine disruption (Edwards and Hamlin 2018). The authors cite other studies that found evidence of deleterious nitrogen effects to freshwater mussels: reduced luring behavior, energy reserves, and embryo production from endocrine disruption (Jobling et al. 2003; Leonard et al. 2014); sensitivity of *L. siliquoidea* and *L. fasciola* to ammonia (Wang et al. 2007); increased nitrates correlated with reduced maximum life span in *Margaritifera margaritifera* (Bauer 1992); and reduced probability of presence of 5 species of freshwater mussels within reaches of a Czech river with elevated nitrate concentrations, e.g., above 4.3 mg/L NO₃-N (Douda 2010). Kwan et al. (2018) studied hemolymph qualities of instar juveniles of horseshoe crabs, and found that nitrate and lead levels impacted amebocyte viability and morphology. The authors noted that nitrate may have negative impacts on hemocyanin, “related to innate immunity, physiological and homeostatic processes in invertebrates, such as phenoloxidase activity (Nagai et al. 2001), agglutination of microorganisms (Alpuche et al. 2010), anti-viral defense (Lei et al. 2008), molting (Adachi et al. 2005), hormone transport (Jaenicke et al. 1999), protein storage and osmoregulation (Paul and Pirow 1998).” (Kwan et al. 2018).

Nobles and Zhang (2015) studied the effects of a municipal wastewater treatment plant on both a native freshwater mussel species and nonnative freshwater Asian clam species placed at varying distances away from the plant outfall. They found physiological effects in these species at least 3.65km from the effluent outfall, as compared to a control site above the outfall, with growth and survival significantly lower below the outfall, though improving with distance from it. They only saw juvenile recruitment at the control site upstream of the effluent discharge.

We found evidence of negative nutrient effects on freshwater shellfish species for a variety of life stages. Endocrine disruption by elevated nutrient levels was of particular problem at all life stages, with reduced: embryo production, recruitment, glochidia stage and metamorphosis success, and

respiration and filtration in adult stages. These effects led to reduced growth and survival of the studies species.

Estuarine invertebrates, including mussels

Similar to studies on freshwater macroinvertebrates, most studies on estuarine invertebrates we reviewed found indirect effects on communities, specifically from algal blooms and associated oxygen fluctuations, development of large algal mats, and other shifts in algal communities. These changes in water chemistry are thought to cascade up the food web through bacterial activity (and oxygen fluctuations), food quality changes from altered primary producer communities, affecting nanoplankton, zooplankton, and macroinvertebrates (Capriulo et al. 2002). The end results observed are dominance by fewer taxa, reduced functional diversity, and overall reduced abundance and density of estuarine invertebrates (Hale et al. 2018; Lawes et al. 2018; Jones et al. 2006).

In a review of aquatic biota sensitivity to nitrate, Camargo et al. (2005) recommends a criterion of <10mg/L overall for invertebrates, fish and amphibians, but notes that 2.0mg/L would be better for freshwater species, and up to 20 mg/L could be acceptable for saltwater species, while noting that early life stages of salt water species can be as sensitive as freshwater species. The most toxic effects of nitrogen enrichment noted by the authors is the conversion of oxygen-carrying pigments into malfunctioning forms, reducing oxygen uptake by aquatic organisms (Camargo et al. 2005; Kwan et al. 2018). In laboratory studies (Camargo et al. 2005; Muir et al. 1991) examined Asian tiger shrimp responses to levels of NO₃-N. At 0.226 mg/L effects were lethal to 31-37% of individuals in the protozoa stage (1st stage), increasing to 37-58% at 0.22 mg/L (Table A5). Most levels used in these studies were well above ambient values, (145-3894 mg/L) and were lethal to juveniles.

Lawes et al. (2018) found that invertebrate larval recruitment and settlement is affected by enriched sediment-bound and/or ambient nutrient levels. Invertebrate taxa groups responded differently to enriched conditions, which is posited to be related to differential signaling pathways, nutrient effects on those pathways, and associated responses (Figure 1). There were fewer recruits overall in enriched conditions, suggesting nutrient levels affected both bacterial communities within surface biofilms (reduced densities), and attractiveness of the surface to settling larva. Bacterial cells had reduced densities when developed under nutrient enriched conditions, which then led to differential settlement responses by larval invertebrates. Seitzinger and Sanders (1997) similarly found a negative correlation between nitrogen enrichment (via DON in this case) and bacterial abundances. In Lawes et al. (2018), serpulid (tubeworm) and mussel colonization have been correlated with biofilm bacterial density, and reduced serpulid recruitment was detected under enriched conditions. Increased barnacle and ascidian (sea squirt) settlement under increased sediment-bound (biofilm) and ambient nutrient levels, respectively, was suggested to be related to positive detection cues in their settlement signaling pathways. The authors suggest that, as is the case with any filter feeding group, the filter feeding capabilities of these taxa could actually be a water quality benefit under certain conditions, but that these taxa could also end up dominating enriched communities. In addition to serpulids, sponge and bryozoan settlement was suppressed under nutrient enriched conditions, which was suggested to be related to either inhibitory settlement cues, or a lack of positive detection cues that are present in the ascidian and barnacle taxa (Lawes et al. 2018).

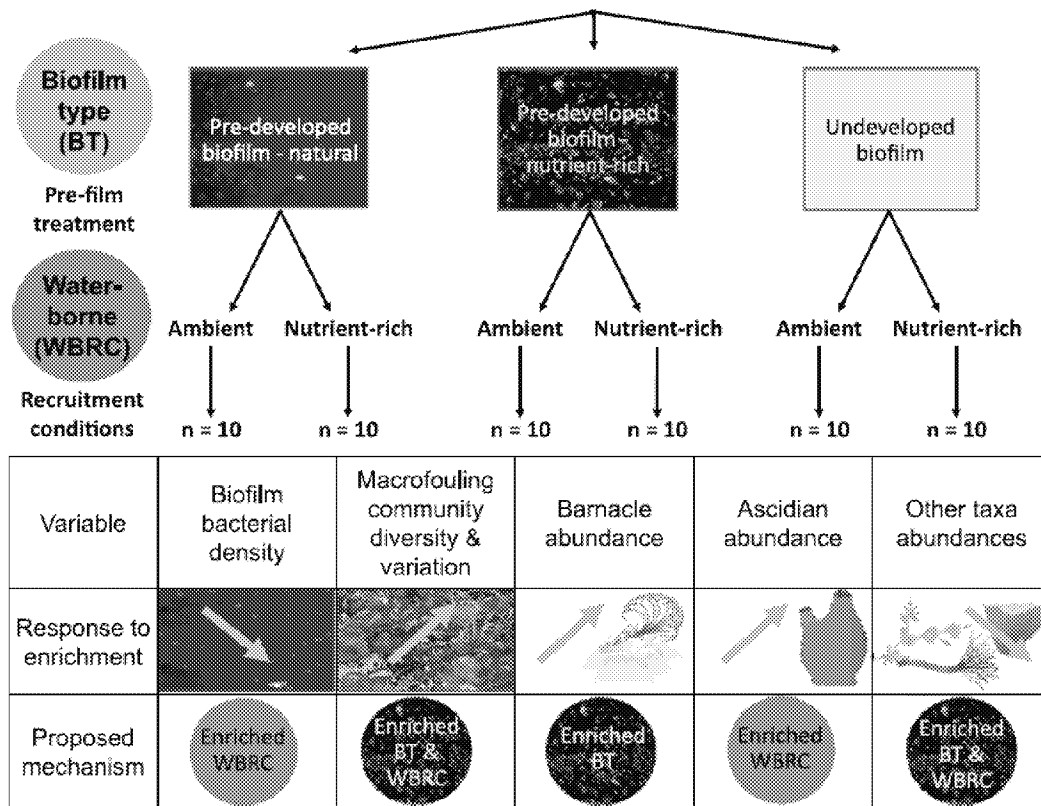


Figure 2. Graphical abstract of study results by Lawes et al. 2018. Nutrients were enriched using 10g of a slow-release fertilizer within a tube surrounding experimental settlement plates. The fertilizer (Osmocote) contains 17.7% N (2.5% ammonium, 13.6% urea, 1.7% nitrate), 2% P (1.6% water soluble P, 0.4% citrate soluble P), and 5.8% K (sulphate form), as well as other trace components. This method was shown to increase local ambient concentrations of TN and TP, with negligible ambient concentrations of other components (Lawes et al 2016).

Several studies have documented shifts in community structure to more nutrient-tolerant taxa. In an estuarine wetland, Ahmadi et al. (2011) found more pollution tolerant indicator species (e.g., gastropods and chironomids) at the more freshwater, nutrient rich sites they studied than at the saline, oligotrophic sites, which had more sensitive species (e.g., mayflies, aquatic beetles) and higher overall invertebrate diversity. In healthy systems, the pattern tends to be reverse, with more invertebrate diversity in freshwater sites than estuarine. The authors found that salinity and freshwater inflow to the wetlands – affecting salinity and nutrient concentration – are key factors affecting the community dynamics and abundances.

Hale et al (2018) looked at temporal shifts in a New England estuary benthic macrofaunal community with changing nutrient dynamics. They found a steady (though non-significant) increase in taxonomic distinctness – a measure of biodiversity that assesses taxonomic tree path length between species – in response to a 50% reduction in TN input from wastewater treatment facilities. Spatial patterns of taxonomic distinctness were related to the north-south declining pollution gradient with distance from human population centers. Patterns of species composition was shown to be correlated with patterns of eutrophication, hypoxia, and sediment contamination. Community composition also related to reduction in phytoplankton corresponding to increasing water temperatures, reducing organic matter

input to benthos, shifting dominance from *Asterias* starfish to Cancer crabs, and fish from primarily demersal to pelagic.

In Portugal, Lillebø et al. (1999) studied European mudsnail (*Hydrobia ulvae*) populations associated with eelgrass bed habitat and red and green algal populations, and along a gradient of eutrophication. While the snail population was stable at the eel grass meadows, abundance was greater during the green algal bloom (associated with high eutrophication), suggesting these snails are tolerant to nutrient enrichment, and perhaps prefer the consistent source of food associated with the blooms. Similar dynamics may be found with algal grazers in the Delaware Estuary, but clearly taxa that benefit from algal blooms are among the minority of those present.

Breitburg et al. (1999) studied mesocosm dynamics to examine the density, abundance and growth patterns under increasing trophic complexity within mesocosms for oysters, fish, clams (*Macoma*) and anemones. They found that the mollusk (oyster and *Macoma* clam) growth was limited by the P and N in the summer, and that they had lower mortality and higher growth rates than other taxa studied. The authors found significantly higher production by algae, bacteria, nanoflagellates, copepods, copepodites and fish densities with additions of N and P. Life stages or growth may have been altered, with reduced amounts of small copepods and reduced amounts of large copepodites. Anemones suffered due to loss of food sources through competition with fish. These 28-day experiments show a bottom-up effect that benefits several trophic levels, but we might presume this effect would not be stable in a natural system. The authors found oyster growth was more dependent on the assemblage of the mesocosm and ambient nutrient levels than on phytoplankton production or particulate nitrogen or carbon.

In a study on multiple anthropogenic stressors on estuarine algae, Fowles et al. (2018) found that elevated nutrients near fish farms and sewage outfalls allow some native algae to colonize nearshore rocky reefs, but non-natives are also supported in high densities near pollution sources, especially those that are tolerant and have shorter life histories than natives. The study suggests a shift from native algae-dominated reefs to more opportunistic, non-indigenous species with increased tolerance, reduced competition from natives.

Phytoplankton algal blooms associated with eutrophication not only deplete dissolved oxygen during the die off, but can affect the co-occurring organisms through trophic mechanisms. Livingston (2007) found a correlation between orthophosphate and ammonia loading and phytoplankton population levels, with high loading inducing blooms. A significant bloom in *Prorocentrum cordatum* was associated with a decline phytoplankton and invertebrate species richness, as well as reduced fish and invertebrate trophic diversity, indicating both top-down and bottom-up effects of nutrient enrichment on the local food web, though the author noted water temperature and salinity, climate conditions, and nutrient loading timing are also affecting the studied organisms (Livingston 2007). In developing an index for brown tide impact, Gastrich and Wazniak (2002) reviewed studies showing that increased bloom-associated phytoplankton cell densities were associated with negative effects in clam, mussel and scallop populations: decreased juvenile and adult growth, feeding, and grazing rates, with increased juvenile and adult mortality rates. Alternatively to Livingston's (2007) findings, Gastrich and Wazniak saw reduced zooplankton and copepod production associated with increased phytoplankton densities. In assessing macroalgal mats effects on an estuarine food web, Raffaelli (1999) found reduced densities of amphipods (*Corophium*), mollusks (*Macoma*) and polychaetes

(Nereis) associated with mat presence. The author suggests this is due to the eutrophication of the water, the non-energetic organic matter input by the algae, and the longer-term effects the algal mats have on the sediment habitability by invertebrates. In an effort to understand the effects of climate change, Najjar et al. (2000) reviewed sources that showed increased particle loads could disrupt filter feeders, and increased nutrients causing algal blooms would decrease light availability for SAV, reducing that important habitat.

The literature shows very few studies showing direct effects of nitrogen and phosphorus compounds on invertebrates in freshwater and estuarine environments, with most effects from indirect pathways as described above. Although several studies have found that macroinvertebrate communities may not be the best indicators for nutrient enrichment, Smith & Tran (2010) refute this statement by utilizing metrics that are more closely related to nutrient concentrations. Few studies note specific nutrient concentrations as change points for the communities or metrics. Those studies that examine levels similar to those found in the Delaware estuary show ranges from values range from 0.18-9.8 mg/L NO₃-N (Sunderman et al. 2015), 1.2-3.3 mg/L TN (Smith & Tran 2010; Sunderman et al. 2015), 0.009-0.27 mg/L TP (Smith & Tran 2010) and 0.01-0.09 mg/L Orthophosphorus (Baker & King 2010; Smith & Tran 2010; Kail et al 2012). The values proposed by Smith & Tran are close to our region and therefore are based on similar fauna, and were developed for large rivers, which has been a difficult study type to find even in other regions.

Algae Impacts and Endpoints

Primary producers are important components for aquatic habitats affecting ecological aspects such as food web and community structure, biogeochemical cycling, and bioaccumulation of environmental contaminants (Hill et al., 2009). Stream biofilms play a large ecological role as a dominant and high-energy food source for upper trophic levels, such as fish and invertebrates (Horne & Goldman, 1994; Allan & Castillo, 2007) while showing quick responses to environmental changes, allowing them to integrate variations in water quality (Lowe and LaLiberte, 2007; Tien et al., 2009). Phosphorus and nitrogen commonly limit primary production in various aquatic systems (Dodds et.al., 2002) that can lead to changes in species composition (Dodds and Welch, 2000; Pedersen & Borum, 1996; Valiela et al., 1997), algal productivity (Correll, 1998) and eutrophication events (Correll, 1998; Correll 1999) and effects on grazers (Cummins et al., 2004; Hauxwell et al., 1998). Although, biofilms are still influenced by current, temperature, light, nutrients, and grazing rates (Horne & Goldman, 1994; Allan & Castillo, 2007).

Many studies have shown estuaries to be more influenced by nitrogen inputs than phosphorus however robust data on nutrient endpoints is largely lacking among various environments. Pedersen & Borum (1996), determined nitrogen concentrations required for phytoplankton growth (37.3 mg-N/g-DW/L) and four species of macroalgae (1.2-6.9 mg-N/g-DW/L) in a Denmark estuary system. Whereas, Yang et al. (2016) determined dissolved inorganic nitrogen (DIN) concentrations for four phytoplankton species in a Chinese estuary (0.28, 0.29, 0.48 mg-N/L for *Chaetoceros curvisetus*, *Ditylum brightwelli* and *Skeletonema costatum*, respectively; Table A6). This study also showed the DIN and TN endpoint to increase with a decrease or increase in salinity due to its effect on algal growth, metabolism and nutrient uptake (Yang et al., 2016). Twilley et al. (1985), determined nitrogen loading rates of ≥ 0.84 mg-N/L/week (0.19 mg-P/L/week of phosphorus) to result in a significant decrease in the submerged plants of *Potamogeton perfoliatus* and *Ruppia maritirna*.

In streams, phosphorus is the main nutrient found to control production leading to eutrophication events (Table A6). Stevenson et al. (2008) determined total phosphorus (TP) threshold concentrations for Mid-Atlantic streams of 0.010-0.012 mg-P/L relative to changes in stream benthic algae and species richness in 607 streams in the mid-Atlantic region, whereas Dodds et al. (1998) determined threshold concentrations of 0.025-0.075 mg-P/L relative to changes in trophic status across 200 various streams across North American and New Zealand. Streams sampled in Kentucky-Indian region and Michigan showed naturally low productivity concentrations of 0.010 mg TP/L and 0.40 mg TN/L but suggested concentrations of TP and TN to be <0.030 mg/L and <1.0 mg/L, respectively, to prevent nuisance blooms (Stevenson et al., 2006). Another study from Clark Fork River, Montana also expressed TP and TN concentrations to be below 0.030 mg/L and 0.35 mg/L, respectively (Dodds et al., 1997). A review paper done by Dodds and Welch (2000), determined TP and TN endpoints to be 0.055 mg/L and 0.25 mg/L, respectively, for an average biomass across a wider range of environments. This evidence of limited ability to translate endpoints developed for one region in another limits the utility of these studies to the Delaware River and Estuary system.

There is a general lack of evidence for nutrient endpoints for specific species from various environments. Overall nutrient loading generally leads to taxonomic changes or harmful algae blooms in aquatic systems. In estuaries, macroalgae and phytoplankton communities have been shown to overtake seagrasses through reduced light availability by over-shading of epiphytes on the seagrass leaves and differences in physiological capabilities (Cummins et al., 2004; Hauxwell et al., 1998; Valiela et al., 1997). These species compositional changes can be dependent on the taxonomy of the environment, residence timing of the water and grazer communities (Valiela et al., 1997). As seen in Twilley et al. (1985), the submerged plant of *Potamogeton perfoliatus* was less affected by decreased light attenuation than *Ruppia maritima* and more complex macroalgae can sustain growth longer off their internal nutrient stores compared to phytoplankton (Pedersen & Borum, 1996). In stream systems, benthic communities have been shown to respond with species taxonomic changes (one diatom species to another) (Carrick et al., 1988; Winter and Duthie, 2000; Ponder et al., 2007), decreased diversity and abundance (Carrick et al., 1988; Dodds and Welch, 2000) and a community structure change to Chlorophyta or Cyanobacteria. *Cladophora* is a common nuisance species that overtakes other algal communities at higher nutrient concentrations (Biggs et al., 2000; Stevenson et al., 2006).

In addition to nutrient impacts on specific species, it is also important to consider algal biomass levels in order to prevent nuisance blooms or eutrophication. In estuaries, macroalgae biomass or phytoplankton biomass are quantified by chlorophyll-a concentrations. Boyer et al. (2009) used water column chlorophyll-a for phytoplankton biomass in Florida estuaries, as a possible bloom indicator, determining the range among sites to be 0.526-2.863 ppb. Green et al. (2014), determined a macroalgae biomass of 15-110 g dm/m² as a resilience threshold in California estuaries, a literature search provided here determined a range of 90-185 g dm/m². Another study in California used the aRPD (apparent Redox Potential Discontinuity) to set their macroalgae criteria to 175 g dw/m² as an exhaustion threshold and determined a literature search range of 110-863 g dw/m² (Sutula et al., 2014). However, in stream environments the common parameter used as a biomass proxy is the benthic algal chlorophyll-a. Many studies across the US set the criteria as less than <100 mg/m² for most systems as biomass levels of ≥100 mg/m² will begin to show increases in filamentous algae (Dodds et al., 1997; Dodds et al., 1998; Welch et al., 1988).

Overall, consistent, quantified nutrient endpoints are lacking for a wide range of aquatic systems, especially estuaries. More data on estuarine impacts and associated endpoints would need to be acquired in order to develop a suggested tolerance range or value. There is also a lack of nutrient endpoints for specific algal species, ranging from macro to microalgae. Most studies do use specific species, but they are usually the most abundant for that area of study and there may not be enough studies per species to make an appropriate nutrient range estimate; more data would need to be collected and reviewed for better species specific requirements. There was a lack of available resources for Pennsylvania estimates and the North-Eastern US in general. This can create a limitation in comparing nutrient endpoints or even utilizing endpoints determined by other studies because most regions can not directly relate to one another due to aquatic habitats experiencing a wide range of environmental influences from natural to human.

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NOTE: This is a comprehensive list of all literature consulted during the process of identifying potential nutrient impacts and relevant concentration thresholds.

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Appendix A: Data Tables

Table A1. Median concentrations in the Delaware Estuary Zones based on the DRBC Boat Run data, 2000-2018.

Zone	Temp. (°C)	Salinity (‰)	TN (mg/L)	NH ₄ (mg-N/L)	NO ₃ + NO ₂ (mg-N/L)	PO ₄ (mg-P/L)
2	16.40	0.11	1.18	0.072	0.89	0.053
3	16.56	0.11	1.35	0.051	1.01	0.063
4	16.61	0.16	1.91	0.068	1.60	0.078
5	16.77	0.79	1.83	0.034	1.52	0.078
6	16.52	16.55	0.69	0.056	0.50	0.043

Table A2. Nutrient concentrations, temperatures and salinities (when available) associated with lethal and non-lethal effects on fish species and communities.

Species	Size/Stage	Nitrogen species (mg/L)				Phosphorus species (mg/L)		Temp (°C)	Salinity (‰)	pH	Description	Reference
		TN	NO ₃ -N	NO ₂ -N	NH ₄ -N	TP	PO ₄					
<i>Carpiodes cyprinus</i>	-	-	-	100*	-	-	-	-	-	-	No mortality in 36 hr	McCoy 1972 as cited in Russo & Thurston 1977
<i>Catostomus commersoni</i>	-	-	-	100*	-	-	-	-	-	-	No mortality in 48 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Cottus bairdi</i>	5.2-5.4	-	-	0.82-8.75	-	-	-	12.8-13.1	Fresh	8.1	No mortalities in 96 hrs	Russo & Thurston 1977
<i>Cottus bairdi</i>	5.2-5.4	-	-	21.6-66.7	-	-	-	13.6	Fresh	8.08	No mortalities in 154 hrs	Russo & Thurston 1977
<i>Cottus bairdi</i>	5.2-5.4	-	-	8.41-26.3	-	-	-	13.2	Fresh	8.14	No mortalities in 72 hrs	Russo & Thurston 1977
<i>Culaea inconstans</i>	-	-	-	5*	-	-	-	-	-	-	Mortality in 3-5 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Cyprinus carpio</i>	-	-	-	100*	-	-	-	-	-	-	Mortality in 45 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Cyprinus carpio</i>	-	-	-	40*	-	-	-	-	-	-	No mortality in 48 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Cyprinella spilopterus</i>	-	-	-	20-40*	-	-	-	-	-	-	Survived less than 12-24 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Etheostoma nitrum</i>	-	-	-	20-40*	-	-	-	-	-	-	Survived less than 12-24 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Gambusia affinis</i>	adult, female	-	-	1.6*	-	-	-	21-24	-	7.1-7.5	24 hr LC50	Wallen et al. 1957 as cited in Russo & Thurston 1977
<i>Hypentelium nigricans</i>	-	-	-	20-40*	-	-	-	-	-	-	Survived less than 12-24 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Ictalurus melae</i>	-	-	-	100*	-	-	-	-	-	-	Mortality in 24 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Ictalurus melae</i>	-	-	-	40*	-	-	-	-	-	-	No mortality in 48 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Ictalurus punctatus</i>	40g	-	-	7.5*	-	-	-	21	-	7.4-7.8	96 hr LC50	Konikoff 1973 as cited in Russo & Thurston 1977
<i>Ictalurus punctatus</i>	fingerlings	-	-	13*	-	-	-	30	-	8.6-8.8	96 hr LC50	Colt 1974 as cited in Russo & Thurston 1977
<i>Lepomis gibbosus</i>	-	-	-	20-40*	-	-	-	-	-	-	Survived less than 12-24 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Lepomis machrochirus</i>	-	-	-	20-40*	-	-	-	-	-	-	Survived less than 12-24 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Morone chrysops</i> x <i>Morone saxatilis</i>	-	-	-	>100	-	-	-	25	8 g/L	6-8	96 hr LC50	Weirich et al. 1993
<i>Morone chrysops</i> x <i>Morone saxatilis</i>	-	-	-	12.8±1.6	-	-	-	25	Fresh	6-8	96 hr LC50	Weirich et al. 1993
<i>Morone chrysops</i> x <i>Morone saxatilis</i>	-	-	-	35.0±2.3	-	-	-	25	1 g/L	6-8	96 hr LC50	Weirich et al. 1993
<i>Noturus flavus</i>	-	-	-	20-40*	-	-	-	-	-	-	Survived less than 12-24 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	100g	-	-	0.55*	-	-	-	10	-	7.9	55% mortality in 24 hrs	Smith & Williams 1974 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	11.8-23.6cm	-	-	0.17-0.41	-	-	-	9.7-10.7	-	7.58-8.33	72 hr LC50	Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	12-14g	-	-	0.19-0.27*	-	-	-	11.6-12.6	-	7.9	96 hr LC50	Russo et al. 1974 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	12g	-	-	0.14-0.14*	-	-	-	12.4	-	7.9	Asymptotic LC50 (8-19 days)	Russo et al. 1974 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	16.7cm	-	-	0.46	-	-	-	10.4	1.2 mg/l	7.92	96 hr LC50	Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	17.0cm	-	-	2.36	-	-	-	10.4	5.1 mg/l	8.01	96 hr LC50	Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	17.6cm	-	-	3.54	-	-	-	10.4	10.4 mg/l	7.9	96 hr LC50	Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	18.4cm	-	-	6.69	-	-	-	10.5	20.2 mg/l	7.84	96 hr LC50	Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	19.4cm	-	-	12.2	-	-	-	10.3	40.9 mg/l	7.74	96 hr LC50	Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	20.0cm	-	-	12.5	-	-	-	10.4	40.8 mg/l	7.69	96 hr LC50	Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	235g	-	-	0.2*	-	-	-	9.5	-	7.9	96 hr LC50	Russo et al. 1974 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	2g	-	-	0.39*	-	-	-	10.8	-	7.9	96 hr LC50	Russo et al. 1974 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	4.5g	-	-	1.6*	-	-	-	10	-	7.9	24 hr LC50	Russo et al. 1974 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	9g	-	-	0.23*	-	-	-	12	-	6.4-6.7	96 hr LC50	Brown & McLeay 1975 as cited in Russo & Thurston 1977
<i>Oncorhynchus mykiss</i>	alevin	-	-	-	0.05	-	-	-	Fresh	-	72 day LC0.01	Camargo & Alonso 2006
<i>Oncorhynchus mykiss</i>	alevin	-	2.3	-	-	-	-	-	-	-	30 day LOEC	Camargo & Alonso 2006
<i>Oncorhynchus mykiss</i>	alevin	-	1.1	-	-	-	-	-	-	-	30 day NOEC	Camargo & Alonso 2006
<i>Oncorhynchus mykiss</i>	alevin	-	-	0.01-0.04	0.16-0.37	-	-	-	Fresh	-	96 hr LC50	Camargo & Alonso 2006
<i>Perca fluviatilis</i>	fry	-	-	-	0.29	-	-	-	Fresh	-	96 hr LC50	Camargo & Alonso 2006
<i>Percina caprodes</i>	-	-	-	5*	-	-	-	-	-	-	Mortality <3 hrs	McCoy 1972 as cited in Russo & Thurston 1977
<i>Phoxinus laevis</i>	5-8cm	-	-	10*	-	-	-	18-21	-	-	Fatal in 14 days	Klingler 1957 as cited in Russo & Thurston 1977
<i>Pimephales promelas</i>	6.2-6.4cm	-	-	2.3-3.81	-	-	-	12.7	Fresh	8.04	96 hr LC50	Russo & Thurston 1977
<i>Pimephales promelas</i>	6.2-6.4cm	-	-	2.37-7.95	-	-	-	13	Fresh	8.05	72 hr LC50	Russo & Thurston 1977
<i>Pimephales promelas</i>	fry	-	-	2.3-3.0	-	-	-	-	Fresh	-	96 hr LC50	Camargo & Alonso 2006
<i>Rutilus rutilus</i>	fry	-	-	-	0.35	-	-	-	Fresh	-	96 hr LC50	Camargo & Alonso 2006
<i>Semotilus atromaculatus</i>	3-4 inch	-	-	80-400*	-	-	-	15-21	-	8.3	Critical range	Gillette et al. 1952 as cited in Russo & Thurston 1977
Fish community	-	1.37	-	NA	-	0.17	-	-	Fresh	-	Effects on IBI/habitat model low order, freshwater streams	Miltner & Rankin 1998

Table A3. Nutrient concentrations, temperatures and salinities (when available) associated with lethal and non-lethal effects on non-shellfish macroinvertebrates species, communities, and community metrics.

Species	Size/Stage	Nitrogen species (mg/L)				Phosphorus species (mg/L)		Temp (°C)	Salinity (‰)	pH	Description	Reference
		TN	NO ₃ -N	NO ₂ -N	NH ₄ -N	TP	PO ₄					
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Early instar larvae	-	6.7	-	-	-	-	-	fresh	-	120 LC0.01	Camargo et al 2005, Camargo and Ward 1995
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Last instar larvae	-	9.6	-	-	-	-	-	fresh	-	120 LC0.01	Camargo et al 2005, Camargo and Ward 1995
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Early instar larvae	-	106.5	-	-	-	-	-	fresh	-	120h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Last instar larvae	-	119	-	-	-	-	-	fresh	-	120h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Early instar larvae	-	191	-	-	-	-	-	fresh	-	72h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Last instar larvae	-	210	-	-	-	-	-	fresh	-	72h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Early instar larvae	-	113.5	-	-	-	-	-	fresh	-	96h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Cheumatopsyche pettiti</i>)	Last instar larvae	-	165.5	-	-	-	-	-	fresh	-	96h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Early instar larvae	-	4.5	-	-	-	-	-	fresh	-	120 LC0.01	Camargo et al 2005, Camargo and Ward 1995
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Last instar larvae	-	6.5	-	-	-	-	-	fresh	-	120 LC0.01	Camargo et al 2005, Camargo and Ward 1995
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Early instar larvae	-	65.5	-	-	-	-	-	fresh	-	120h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Last instar larvae	-	77.2	-	-	-	-	-	fresh	-	120h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Early instar larvae	-	148.5	-	-	-	-	-	fresh	-	72h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Last instar larvae	-	183.5	-	-	-	-	-	fresh	-	72h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Early instar larvae	-	97.3	-	-	-	-	-	fresh	-	96h LC50	Camargo et al 2005, Camargo and Ward 1992
Net-spinning caddisfly (<i>Hydropsyche occidentalis</i>)	Last instar larvae	-	109	-	-	-	-	-	fresh	-	96h LC50	Camargo et al 2005, Camargo and Ward 1992
Water flea (<i>Ceriodaphnia dubia</i>)	Neonates (<24hr)	-	374	-	-	-	-	-	fresh	-	48h LC50	Camargo et al 2005; Scott and 2000
Water flea (<i>Ceriodaphnia dubia</i>)	Neonates (<24hr)	-	7.1-56.5	-	-	-	-	-	fresh	-	7d NOEC	Camargo et al 2005; Scott and 2000
Water flea (<i>Ceriodaphnia dubia</i>)	Neonates (<24hr)	-	14.1-113	-	-	-	-	-	fresh	-	7d LOEC	Camargo et al 2005; Scott and 2000
Water flea (<i>Daphnia magna</i>)	Neonates (<48hr)	-	462	-	-	-	-	-	fresh	-	48h LC50	Camargo et al 2005; Scott and 2000
Freshwater community	-	3.37+/-1.1	0.18-0.98	-	1.37+/-1	-	-	-	fresh	-	-	Sunderman et al., 2015
Nutrient Biotic Index, Pollution Tolerant Index, % eutrophic taxa, % mesotrophic taxa	-	1.2	0.55	0.18-0.55	-	0.009-0.27	-	-	fresh	-	Large rivers	Smith & Tran, 2010
Freshwater community	-	-	-	-	0.03-0.08	-	-	-	-	-	-	Kail et al., 2012
Estuary community	-	-	-	-	-	-	-	-	0.5-31.8	6.7-8.1	-	Sharp et al., 1982
Freshwater community	-	-	-	-	-	-	0.01-0.09	-	-	-	-	Kail et al., 2012; Baker & King 2010, Smith & Tran 2010
EPT abund	-	-	-	-	-	-	0.33mg/L DRP	-	fresh	-	Not threshold	Elbrecht et al 2016
Baetis spp	-	-	-	-	-	-	0.33mg/L DRP	-	-	-	-	Elbrecht et al 2016
Gammarus spp	-	-	-	-	-	-	0.33mg/L DRP	-	-	-	-	Elbrecht et al 2016
IBI	-	-- No relationship btw IBI & nutrients in large rivers --						-	-	-	-	Miltner et al 1998
EPT relabund	-	0.59	-	-	-	0.021	-	-	-	-	Watersheds <165km ² in S Ontario, <500-700 km ² in N.	Chambers et al 2012
EPT richness	-	2.83	-	-	-	0.021	-	-	fresh	-	-	Chambers et al 2012
Family-Level Biotic Index	-	2.08	-	-	-	0.024	-	-	-	-	-	Chambers et al 2012
Diptera + non-insect	-	2.08	-	-	-	0.063	-	-	-	-	-	Chambers et al 2012

Table A4. Results from nonparametric change point analysis in non-tidal rivers using only biological water-quality metrics that were significantly correlated with primary nutrient criteria variables. Threshold responses for the 5th, 50th, and 95th percentile of cumulative probabilities are given. Probability of Type I error (p) is for 50th percentiles only. Mean scores at sites with nutrient concentrations below or above the 50th percentile are provided for each water-quality metric. Adapted from Smith & Tran (2010).

Primary nutrient criterion variable	Water quality metric	50th percentile	Below-50th % (Non-impaired)	Average (above + below /2)	Above-50th % (Impaired)
TP (mg/L)	NBI-P	0.011	5.4	6.45	7.5
	% mesotrophic	0.03	30	17	4
	% eutrophic	0.009	27	46	65
TN (mg/L)	NBI-P	0.51	5.9	6.75	7.6
	% mesotrophic	0.41	25	15	5
	% eutrophic	0.5	30	47.5	65
	PTI	1.22	2.7	2.5	2.3
NO ₃ -N (mg/L)	NBI-P	0.18	6	6.9	7.8
	PTI	0.55	2.7	2.5	2.3
Chl a (mg/m ³)	HBI	2.1	5.3	6.1	6.9

TP = total phosphorus, TN = total nitrogen, Chl a = chlorophyll a, NBI-P = [Macroinvertebrate] Nutrient Biotic Index-P, % mesotrophic = % mesotrophic individuals (diatoms), % eutrophic = % eutrophic individuals (diatoms), PTI = [Diatom] Pollution Tolerance Index, HBI = Hilsenhoff's [Macroinvertebrate] Biotic Index

Table A5. Nutrient concentrations, temperatures and salinities (if available) associated with lethal and non-lethal effects on best available shellfish species, communities, and community metrics.

Species	Size/Stage	Nitrogen species (mg/L)				Phosphorus species (mg/L)		Temp (°C)	Salinity (‰)	pH	Description	Reference
		TN	NO ₃ -N	NO ₂ -N	NH ₄ -N	TP	PO ₄					
Eastern oyster	Juveniles	-	3794	-	-	-	-	-	seawater	-	96 h LC50	Camargo et al 2005; Epifano and Srna 1975
Marine prawns (incl. <i>P. aztecus</i> , <i>P. schmitti</i> , <i>P. setiferus</i>)	Juveniles	-	3400	-	-	-	-	-	28	-	48 h LC50	Camargo et al 2005; Wickins 1976
Asian tiger shrimp	Protozoa (I stage)	-	0.226	-	-	-	-	-	32	-	31-37% mortality 40h	Camargo et al 2005; Muir et al 1991
Asian tiger shrimp	Protozoa (I stage)	-	2.26	-	-	-	-	-	32	-	35-43% mortality 40 hr	Camargo et al 2005; Muir et al 1991
Asian tiger shrimp	Protozoa (I stage)	-	22.6	-	-	-	-	-	32	-	37-58% mortality 40 hr	Camargo et al 2005; Muir et al 1991
Asian tiger shrimp	Juveniles (22–35 mm)	-	2876	-	-	-	-	-	15	-	48 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	1723	-	-	-	-	-	15	-	72 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	1449	-	-	-	-	-	15	-	96 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	3894	-	-	-	-	-	25	-	48 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	2506	-	-	-	-	-	25	-	72 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	1575	-	-	-	-	-	25	-	96 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	4970	-	-	-	-	-	35	-	48 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	3525	-	-	-	-	-	35	-	72 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	2316	-	-	-	-	-	35	-	96 h LC50	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	145	-	-	-	-	-	15	-	"safe level"	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	158	-	-	-	-	-	25	-	"safe level"	Camargo et al 2005; Tsai and Chen 2002
Asian tiger shrimp	Juveniles (22–35 mm)	-	232	-	-	-	-	-	35	-	"safe level"	Camargo et al 2005; Tsai and Chen 2002
Australian red claw crayfish	Juveniles (9 - 13 mm)	-	1000	-	-	-	-	-	fresh	-	5d NOAEL	Camargo et al 2005; Meade and Watts 1995
European crayfish	Juveniles	-	14	-	-	-	-	-	fresh	-	7d NOAL	Jensen 1996
Quagga mussel	-	-	-	-	-	-	476	20.47 ± 0.02	fresh	8.32 ± 0.03	96hr LC100 NaPO4	Vijayavel and Kashian 2019
Quagga mussel	-	-	-	-	-	-	260	20.47 ± 0.02	fresh	8.32 ± 0.03	96hr LC50 NaPO4	Vijayavel and Kashian 2019
Quagga mussel	-	-	-	-	-	-	125	20.47 ± 0.02	fresh	8.32 ± 0.03	96hr LC0 NaPO4	Vijayavel and Kashian 2019
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	2009	-	-	-	-	-	fresh	-	24h LC50	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	1128	-	-	-	-	-	fresh	-	24h LC10	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	1297	-	-	-	-	-	fresh	-	48h LC50	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	728	-	-	-	-	-	fresh	-	48h LC10	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	1121	-	-	-	-	-	fresh	-	72h LC50	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	629	-	-	-	-	-	fresh	-	72h LC10	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	1042	-	-	-	-	-	fresh	-	96h LC50	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	585	-	-	-	-	-	fresh	-	96h LC10	Camargo et al 2005; Alonso and Camargo 2003
New Zealand mud snail (<i>Potamopyrgus antipodarum</i>)	Adults (2.6–3.8 mm)	-	195	-	-	-	-	-	fresh	-	96h LC0.01	Camargo et al 2005; Alonso and Camargo 2003

Table A6. Nutrient concentrations associated with effects on freshwater and estuarine algae species and communities.

Species/Parameter	Size/Biomass	Nitrogen species (mg/L)					Phosphorus species (mg/L)		Temp (°C)	Salinity (‰)	pH	Notes	Reference
		TN	DIN	NO ₃ -N	NO ₂ -N	NH ₄ -N	TP	PO ₄					
Benthic algae		-	-	-	-	-	0.010-0.012	-	-	fresh	-	Statistically determined; 607 mid-Atlantic streams	Stevenson et al. 2008
Benthic chlorophyll	< 50 mg/m ²	0.47	-	-	-	-	0.055	-	-	fresh	-		Dodds et al. 1997, as cited in Dodds & Welch 2000
Benthic chlorophyll	< 50 mg/m ²	0.25	-	-	-	-	0.021	-	-	fresh	-		Lohman et al. 1992 as cited in Dodds & Welch 2000
Benthic chlorophyll	< 200 mg/m ²	3.00	-	-	-	-	0.415	-	-	fresh	-	Calculated	Dodds & Welch 2000
Planktonic chlorophyll	< 0.008 mg/L	0.29	-	-	-	-	0.042	-	-	fresh	-	Calculated	Dodds & Welch 2000
Periphyton & macrophytes		-	1.0	-	-	-	0.02	-	-	fresh	-	Control levels, Bow River, Alberta	Dodds & Welch 2000
Phytoplankton		-	-	-	-	-	0.07	-	-	fresh	-	Control levels	Dodds & Welch 2000
Periphyton	< 200 mg/m ²	-	0.019	-	-	-	-	0.002	-	fresh	-		Dodds & Welch 2000
Cladophora	Prevent nuisance	< 1	-	-	-	-	< 0.03	-	-	fresh	7.94-8.04		Stevenson et al. 2006
Primary productivity	Prevent low levels	< 0.4	-	-	-	-	< 0.01	-	-	fresh	-		Stevenson et al. 2006
Trophic status		-	-	-	-	-	0.025-0.075	-	-	fresh	-	Statistically determined; 200 N. American & New Zealand streams	Dodds et al. 1998
Chl-a biomass	< 100 mg/m ²	0.35	-	-	-	-	0.03	-	-	fresh	-	More strongly correlated to TN & TP than DIN & SRP	Dodds et al. 1997
<i>Chaetoceros curvisetus</i>		-	0.28	-	-	-	-	0.033	-	31	-	Ecological response	Yang et al. 2016
<i>Chaetoceros curvisetus</i>		-	0.53	-	-	-	-	0.029	-	25	-	Ecological response	Yang et al. 2016
<i>Chaetoceros curvisetus</i>		-	0.62	-	-	-	-	0.034	-	15	-	Ecological response	Yang et al. 2016
<i>Ditylum brightwellii</i>		-	0.29	-	-	-	-	0.035	-	31	-	Ecological response	Yang et al. 2016
<i>Ditylum brightwellii</i>		-	0.37	-	-	-	-	0.061	-	25	-	Ecological response	Yang et al. 2016
<i>Ditylum brightwellii</i>		-	0.38	-	-	-	-	0.049	-	15	-	Ecological response	Yang et al. 2016
<i>Skeletonema costatum</i>		-	0.43	-	-	-	-	0.056	-	31	-	Ecological response	Yang et al. 2016
<i>Skeletonema costatum</i>		-	0.27	-	-	-	-	0.039	-	25	-	Ecological response	Yang et al. 2016
<i>Skeletonema costatum</i>		-	0.37	-	-	-	-	0.079	-	15	-	Ecological response	Yang et al. 2016
Phytoplankton biomass		-	-	-	-	-	-	-	-	0.2 -25	-	Sequentially limited by P in spring and by N in summer	Fisher et al. 1992
Macroalgae growth & internal N storage		-	-	-	-	-	-	-	-	23-28	-	Respond to N but not P; response varies by species, site &	Teichberg et al. 2008